A Comprehensive Evaluation of Salmonella Microbiology Testing on Pork Product and Application in a Pork Facility

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Introduction

Salmonella is a motile, non-spore forming, Gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. Non-motile variants include Salmonella Gallinarum and Salmonella Pullorum. The genus Salmonella is divided into two species that can cause illness in humans, Salmonella bongori and Salmonella enterica, the latter being characterized as being the greatest public health concern.

Every year, Salmonella is estimated to cause approximately one million foodborne illnesses in the United States, which correlates to approximately 64% of pathogen bacterial foodborne outbreaks. Most people infected with Salmonella develop diarrhea, fever, and abdominal cramps that last 12 to 72 h after infection. The illness usually lasts 4 to 7 days, and most people recover without treatment. However, for some people the diarrhea may be severe enough to require hospitalization.

FSIS microbiological sampling and testing programs are implemented to ensure that establishments maintain control of their production processes and adhere to FSIS regulations, policies and pathogen performance standards. FSIS conducts routine microbiological sampling and testing for select pathogens, including *Salmonella.* Quantification of *Salmonella* is performed using a most probable number (MPN) methodology which can take up to 4-7 days to confirm the presence of *Salmonella* in matrices evaluated.

Escherichia coli is a bacterium that is commonly found in the human gastrointestinal tract. Some strains, however, can be pathogenic to humans: STEC, Enteropathogenic *Escherichia coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), etc. STEC are *E. coli* that produce Shiga-toxins encoded by *stx* genes. All STEC are not necessarily associated with human disease. The term EHEC is used to designate a subset of STEC, which carry the *stx* and *eae* genes. EHEC are considered to be highly pathogenic to humans as they produce Shiga-toxins and cause attachment and effacement lesions on intestinal cells. The infections caused by EHEC are a major public health problem due to the severity of the clinical symptoms, such as hemorrhagic colitis, but especially post-diarrheal hemolytic uremic syndrome (HUS), particularly in young children and the elderly.

Purpose

To evaluate the performance of the GENE-UP SLM (Salmonella detection), GENE-UP Quant Sal (Salmonella enumeration) and GENE-UP EHEC series (STEC detection) against the USDA/FSIS MLG 4.12 (Salmonella) and 5C.02 (E. coli) reference methods in multiple validation studies on pork samples.

Methods

For the detection phase of this study, MicroTally cloth test portions were prepared by separately inoculating at a low and high level 50 g batches of raw pork trim parts with *Salmonella* Abaetetuba NCTC 8244 and *Escherichia coli* O157:H7 ATCC 35150. The matrixes were mixed with 200 mL of BPW to produce a trim/slurry inoculum. The trim/slurries were used to inoculate the MicroTally cloths. The cloths were placed into sterile bags and held for 48-72 h at 2-8°C prior to analysis.

The pork trim sampling clothes were analyzed at three levels of artificial contamination: non-inoculated (0 CFU/test portion), low level (0.2-2 CFU/test portion), and high level (2-10 CFU/test portion). For the control and high level, 5 replicates were evaluated by the candidate and reference method. For the low level, 20 replicates were evaluated by each method.

Candidate method sampling clothes were enriched in BPW and analyzed at multiple time points. Additionally, unpaired reference method test portions enriched in mTSB were evaluated by the candidate method at 8 and 15 h time points. All candidate method test portions, regardless of presumptive results, were culturally confirmed by the FSIS MLG 4.11 method as well as the direct plating alternative procedure.

For the enumeration phase of this study, the surface of raw pork test portions were sampled. The clothes were then inoculated with the target microorganism at three different levels. The cloth was stored for 48-72 h at refrigerated temperature (2–8°C). After equilibration, MicroTally cloths were analyzed by the candidate method and reference method. Results for carcass sampling cloths are reported as CFU/cloth.

Results

For detection, the study data indicate with 95% confidence that the true difference between the candidate (BPW at 10 and 24 h) and reference methods for MicroTally from pork could be as small as -0.32 or as large as 0.23, with a best estimate of -0.05. For MicroTally cloth from pork test portions evaluated using mTSB (8 and 15 h), the study data indicate with 95% confidence that the true difference between the presumptive and confirmed result could be as small as -0.13 or as large as 0.13, with a best estimate of 0.00.

For the enumeration phase, no natural contamination was detected in the screening of the product, therefore, each matrix was artificially contaminated. Statistical analysis was conducted for each contamination level. Logarithmic transformation of the counts (CFU/g, mL or cloth) was performed and the difference of means, with 90% and 95% confidence intervals, between the candidate method and the reference method, were determined for each contamination level. The 90% confidence interval of the bias between the two methods was calculated to see if the methods fell between -0.5 to 0.5 Log_{10} for each concentration to determine equivalence. The 95% confidence interval of the bias between the two methods was calculated to see if the point 0 fell within the range to determine statistical difference between the methods. The repeatability (s_r) calculated as standard deviation (SD), and relative standard deviation (RSD_r) of each method was determined.

The GENE-UP methods are quick and simple to perform, providing results in less than 1.5 h post incubation of the selective enrichment for 30 sample replicates. With ready-to-use lyophilized PCR reagents, it allows the user to conduct PCR without an additional step of adding the master mix, reducing the amount of hands on time during PCR which eliminates the chance of contamination. The GENE-UP software is user friendly with the ability to track lot information and sample identification quickly and with ease.

Significance

All investigated test kits demonstrated reliability as a rapid method for the detection and enumeration of *Salmonella* spp and the detection of pathogenic *E coli* in pork matrices.